Docket No.: 211381US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Mikio TAKAIWA et al. : ATTN: APPLICATION DIVISION

SERIAL NO: NEW APPLICATION :

FILED: HEREWITH :

FOR: ALKALINE PROTEASE

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as shown in the marked-up copy following this amendment.

Page 1, before line 1, please insert

--This application is a Continuation of U.S. Application Serial No. 09/509,814, filed on April 6, 2000, now pending, which is a 371 of PCT/JP98/04528, filed October 7, 1997.--

Page 48 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.

Page 4, replace the paragraph beginning at line 1 with the following paragraph:

--Fig. 3 shows the effects of pH on the stability of alkaline protease KP43 (10°C, 24 hours). Fig. 4 shows the effects of temperature on the activity of alkaline protease KP43. Fig. 5 shows the effects of temperature on the stability of alkaline protease KP43. Fig. 6 shows the effect of an oxidizing agent (50 mM hydrogen peroxide) on the activity of alkaline protease KP 43. Fig. 7 shows N-terminal sequences of KP9860 protease and partially degraded products thereof SEQ ID NOS:9-13). Fig. 8 shows primer sequences (SEQ ID NOS: 14-20) designed from an N-terminal sequence of KP9860 protease (SEQ ID NOS: 9-13). Fig. 9 shows 57 bp PCR-amplified fragments and primer designs (SEQ ID NOS:21-24).--

Page 5, replace the last paragraph with the following paragraph:

--The alkaline protease of the present invention preferably has an amino acid sequence shown in SEQ ID NOS: 1 or 2, or such a sequence in which one or more amino acids are deleted, substituted, or added. SEQ ID NO: 1 differs from SEQ ID NO: 2 in that lysine at the 3rd position in SEQ ID NO: 2 is deleted. Xaa in SEQ ID NOS: 1 and 2 refers to an arbitrary amino acid. Preferable amino acids for Xaa at each position in SEQ ID NO: 2 are shown in the following Table.--

Page 7, replace the paragraph beginning at line 2 with the following paragraph:

--Examples of the alkaline protease include alkaline proteases having an amino acid sequence shown by SEQ ID NOS: 4, 6, or 8, or such a sequence in which one or more amino acids are deleted, substituted, or added.--

Page 11, replace the paragraph beginning at line 9 with the following paragraph:

--Examples of the nucleotide sequence of the alkaline protease of the present invention are shown in SEQ ID NOS: 3, 5 and 7. The nucleotide sequence is not limited to SEQ ID NOS: 3, 5 or 7, and acceptable sequences may include a nucleotide sequence

encoding the amino acid sequence shown in SEQ ID NOS: 1 or 2, and a nucleotide sequence encoding such an amino acid sequence in which one or more amino acids are deleted, substituted, or added. Of these, nucleotide sequences represented by SEQ ID NOS: 3, 5 and 7, or such sequences in which one or more amino acids are deleted, substituted, or added are preferred. In these cases, deletion, substitution, or addition preferably occurs within the above-described variation of amino acid sequence.--

Page 29, replace the paragraph beginning at line 21 with the following paragraph:

--The obtained N-terminal sequences are shown in Fig. 7. (SEQ IDS NOS: 9-13).--

Pages 29-30, replace the last paragraph with the following paragraph:

--20-30 Nucleotides primers (SEQ ID NOS: 14-20 for 5'-terminal of + chain and that of the - chain corresponding to the obtained N-terminal sequences were synthesized (SEQ ID NOS: 9-13). PCR reaction was carried out in a 100-μL reaction system by use of a template DNA (100 ng), a primer (20 pmol), and PwoDNA polymerase (product of Boehringer Mannheim). When inverse PCR was performed, Expand™ long template PCR system (product of Boehringer Mannheim) was used in a 50-μL reaction system. PCR carried out by use of these primers, 9860-N2 SEQ ID NO: 14) and 9860-25k-RV (SEQ ID NO: 17), provided a DNA fragment of 527 bp.--

Page 31, replace the last paragraph with the following paragraph:

--Inverse PCR was performed by use of primers (1~4 (Fig. 9 (SEQ ID NOS: 21-24) Synthesized from the obtained 527 bp sequence. The KP-9860 chromosome was completely digested by use of restriction enzymes, i.e., *Eco*RI, *Hin*dIII, *Pst*I, and *Bgl*II, and each sample was treated by use of Ligation Kit Ver. 2 (product of--.

Page 33, replace the first and second paragraphs with the following:

--(NDVARHIVKADVAQSSYGLY) (SEQ ID NO: 9) which matches the N-terminal sequence of the purified KP9860 protease. Judging from the N-terminal sequence, the muture region of KP9860 protease gene was deduced to be the 1302 bp, encoding 434 amino acid residues (SEQ ID NO: 4), molecular weight 45310 Da). Upstream of the ORF, there were observed sequences which are deduced to be a promoter region (-35 region: ttgtgt, -10 region: tacgat) and a ribosome-binding site (SD sequence: aggagt). Downstream of the termination codon (taa), there was an inverted repeat having a free energy of -26.2 kcal/mol, which is deduced to be a terminator.

The procedure of Example 5 was repeated, to thereby analyze the entire nucleotide sequence and amino acid sequence of each of the genes of KP-43 protease and KP-1790 protease. The results are shown in SEQ ID NOS: 4 and 5.---

IN THE CLAIMS

Please amend the claims as shown in the marked-up copy following this amendment.

--4. (Amended) A gene encoding an alkaline protease according to Claim 1.

5. (Amended) A microorganism producing an alkaline protease according to Claim 1.

6. (Amended) A detergent composition containing an alkaline protease according to Claim 1.--

REMARKS

Claims 1-6 are pending in this application.

This application is a Continuation of U.S. Application Serial No. 09/509,814, filed on April 6, 2000, now pending, which is a 371 of PCT/JP98/04528, filed October 7, 1997.

Applicant submits that the sequence information recorded in the paper copy of the Sequence Listing attached herewith is identical to the computer-readable Sequence Listing filed May 24, 2000 in the parent application.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Page 5, replace the last paragraph with the following paragraph:

--The alkaline protease of the present invention preferably has an amino acid sequence shown [by Sequence No. 1 or 2] in SEQ ID NOS: 1 or 2, or such a sequence in

which one or more amino acids are deleted, substituted, or added. [Sequence No. 1] SEQ ID NO: 1 differs from [Sequence No. 2] SEQ ID NO: 2 in that lysine at the 3rd position in [Sequence No. 2] SEQ ID NO: 2 is deleted. Xaa in [Sequence Nos. 1 and 2] SEQ ID NOS: 1 and 2 refers to an arbitrary amino acid. Preferable amino acids for Xaa at each position in [Sequence No. 2] SEQ ID NO: 2 are shown in the following Table.--

Page 7, replace the paragraph beginning at line 2 with the following paragraph:

--Examples of the alkaline protease include alkaline proteases having an amino acid sequence shown by [Sequence No. 3, 4, or 5] SEQ ID NOS: 4, 6, or 8, or such a sequence in which one or more amino acids are deleted, substituted, or added.--

Page 11, replace the paragraph beginning at line 9 with the following paragraph:

--Examples of the nucleotide sequence of the alkaline protease of the present invention are shown in [Sequence Nos. 3 to 5] SEQ ID NOS: 3, 5 and 7. The nucleotide sequence is not limited to [Sequence Nos. 3 to 5] SEQ ID NOS: 3, 5 or 7, and acceptable sequences may include a nucleotide sequence encoding the amino acid sequence shown in [Sequence No. 1 or 2] SEQ ID NOS: 1 or 2, and a nucleotide sequence encoding such an amino acid sequence in which one or more amino acids are deleted, substituted, or added. Of these, nucleotide sequences represented by [Sequence Nos. 3 to 5] SEQ ID NOS: 3, 5 and 7, or such sequences in which one or more amino acids are deleted, substituted, or added are preferred. In these cases, deletion, substitution, or addition preferably occurs within the above-described variation of amino acid sequence.--

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The procedure of Example 5 was repeated, to thereby analyze the entire nucleotide sequence and amino acid sequence of each of the genes of KP-43 protease and KP-1790 protease. The results are shown in [Sequence Nos. 4 and 5] SEQ ID NOS: 4 and 5.---

IN THE CLAIMS

Please amend the claims as follows.

--4. (Amended) A gene encoding an alkaline protease according to [any one of claims 1 through 3] Claim 1.

5. (Amended) A microorganism producing an alkaline protease according to [any one of claims 1 through 3] Claim 1.

6. (Amended) A detergent composition containing an alkaline protease according to [any one of claims 1 through 3] Claim 1.--